MODIFIED CARBON BASED ELECTRODES FOR ELECTROCHEMICAL SENSING OF BIOMOLECULES

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Abstract

Electrochemical sensors for different biomolecules are developed using modified pencil graphite electrodes. Different modifications like conducting polymers, nanoparticles, graphene coatings, oligosaccharides and metal oxides are used to enhance the electron transfer between pencil graphite electrode (PGE) and analyte and improve their sensitivity to detect the analyte molecules Biomolecules like cholesterol, cortisol, vitamin B6 and morin were analyzed by modified PGE. By the use of characterization techniques such as SEM, TEM, AFM, XRD, XPS and FT-IR the structural and morphological properties of the modified electrode are analyzed. Cyclic voltammetry (CV), electrochemical impedence spectroscopy (EIS) and differential pulsed voltammetry (DPV) were employed for the electrochemical studies and sensing. Optimisation of experimental conditions such as effect of pH, effect of scan rate, potential window and effect of concentration of analytes were studied in detail. Linear dynamic range, detection limit, quantitation limit, selectivity and reproducibility of the methods were also established. The developed methods were successfully applied for the analysis of biomolecules in different real samples.

Keywords: Pencil Graphite electrode, Biomolecules, Graphene, Electrochemical sensors.

TABLE OF CONTENTS

1. Introduction…….. 1

2. Review of Literature……. 5

3. Objectives…….. 8

4. Research work carried out…… 9

5. Conclusions……...23

Bibliography……24

Publications and Proceedings……..32

1. Introduction

Now-a-days a large variety of biological analytes are detected by highly sensitive electrochemical sensors which are inexpensive and simple as compared to conventional methods such as UV-vis absorption spectroscopy, spectrofluorometry, HPLC and gas chromatography. Electrochemical analysis is exceptional owing to its economical, low energy consuming and unique approach in the method design, and high sensitivity for the analyte determination. Carbon based electrochemical sensors are commonly used because of their low cost, good electron transfer kinetics, good chemical stability, and biocompatibility [1,2]. The traditional carbon-based sensors include glassy carbon electrodes, carbon fibers and pyrrolytic graphite [3]. Recently, carbon nanomaterials of size 1 to 100 nm have been incorporated into sensors as they are advantageous owing to their large surface-to-volume ratio and specific surface area [4]. In addition, carbon nanomaterials have enhanced interfacial adsorption properties, better electrocatalytic activity, high biocompatibility, and fast electron transfer kinetics compared to many traditional electrochemical sensors [5]. Polymer coatings can also be used to modify the physical and chemical properties of carbon nanomaterials [6]. Metal oxides and metal nanoparticles are often incorporated into electrodeposited polymers to enhance the electrocatalytic activity [7].

Recently electrochemical properties of pencil graphite electrodes (PGEs) have been explored in the analysis of various organic compounds. High electrochemical reactivity, easy modification, commercial accessibility, fine rigidity, disposability and low-cost of PGE make it ideal to be used as an effective working electrode.

PGEs are also easy to use and there is no tedious electrode surface cleaning/polishing step [8]. Different types of voltammetric techniques are used in order to quantify a variety of analytes from a wide range of samples, using PGEs which showed reproducible signals, yielding well-defined voltammetric peaks. These electrodes have proven to provide good sensitivity and reproducibility, being a viable, renewable, and economical tool [9,10]. In comparison to other working electrodes, modified PGE has lower background current, good adsorption properties, conductivity and ultra level electrochemical sensing ability towards different analytes **[**11**]**. Uniform composition and surface provided by PGE results in a good quality voltammetric signals of analytes.

Graphene is a honey comb network of SP^2 hybridized carbon atoms, with an extraordinary electronic transport property. The uniqueness and versatility of graphene is due to its physical and chemical properties such as high surface area, ease of functionalization and long-range π conjugation. It creates an ideal platform for gas sensors, field effect transistors, electrochemical resonators and bimolecular sensors [12, 13]. The conductivity of graphene is estimated to be about 64 mS/cm, which is approximately sixty times better than that of single walled carbon nanotubes [14]. Graphene possesses excellent electrocatlytic activity and is an apt material for electrode modification.

Decoration of electrodes with polymeric materials by electropolymerization techniques is an efficient strategy in improving the functioning of electrochemical sensors. Polyaniline (PANI), polypyrrole (PPy), poly(3,4 ethylenedioxythiophene) (PEDOT) and polythiophene (PTh) are a few well studied conducting polymers for various electrocatalytic applications **[**15, 16**]**. Exceptional stability, ease of development, high electrical conductivity and redox

properties are the characteristic features which make PANI a versatile conducting polymer in electrocatalytic applications [17].

Cyclodextrin (CD) is a macrocyclic glucose oligomer, consisting generally of six, seven or eight D glucose units resulting in α-, β- or γ- CD, respectively [18]. Among them β-cyclodextrin has been successfully used for electrode modifications. Being a model host site, β-CD is capable of allowing a variety of guest molecules inside their torus like designed cavities. The interaction of molecular cavities with guest molecules has been extensively analyzed with the intention of bringing more clarity to the specific recognition effects and selectivity in the reaction [19].

Recently the efforts were focused on the discovery of electrocatalysts with non-precious metal oxide modifiers [20]. MnO_2 represents a low-cost and environmentally benign metal oxide which had been widely used as the electrocatalyst in many electrochemical reactions. Manganese oxide-phosphate electrochemically deposited on reduced graphene oxide composite has already been reported as an electrocatalyst for the oxygen evolution reactions [21]. Among different metal oxide modifiers ZnO nanoparticles have also attracted much attention in the field of electrochemical sensing due to the distinctive mechanical features and the nontoxic nature [22].

Biomolecules are organic molecules like proteins, carbohydrates, lipids and nucleic acids that occur naturally in living organisms [23]. They will have specific shape and dimensions and their functional group will determine the chemical properties of the compound. Environmental and biomedical industries are very keen on development of biosensors. Accurate, early, fast diagnosis of many diseases requires the direct detection of biomolecules [24]. Therefore, electrochemical study on biomolecules is an appropriate way to tackle the current scenario. Cortisol is a scientifically established and scrutinized stress biomarker. It belongs to the

glucocorticoid hormone family which is usually secreted upon stress and is an active partner involved in the synchronization of different physiological activities [25]. Cortisol controls blood glucose level, reproductive cycles, stress-immune system and carbohydrate metabolism [26]. A very high concentration of cortisol in human body can lead to the Cushing's disease with the symptoms of obesity, fatigue and bone fragility, whereas the low cortisol levels may lead to Addison's disease which is characterized by weight loss, fatigue, and darkening of skin folds and scars [27]. Therefore, it is important to develop highly sensitive sensors to detect this stress hormone for the betterment of health.

Plants, fruits and flowers contain a variety of low molecular weight products that can strengthen the human immune and health system. They are rich in a variety of antioxidant molecules. Flavonoids are one of the antioxidant molecules that belong to a broad class of polyphenyl group containing benzene-γ-pyrone component. Morin (3,2,4,5,7-pentahydroxyflavone) is one of the most common natural flavonoids present in mulberry leaves, almonds, figs, grapes and some of the ayurvedic products [28, 29**]**. It can act as an antiviral, anti-inflammatory and anti-allergic compound in pharmaceutical industry [30]. It functions as a chemo preventive measure against oral carcinogenesis and protective measure for cardio activities [31]. The direct capturing of singlet oxygen family and poisoning of enzymes involved in the formation of reactive oxygen species elucidates the antioxidant feature of morin. Literature study shows that not many electrochemical methods are available for the analysis of morin.

Determination cholesterol in blood samples is one of the most generally performed laboratory tests. Cholesterol which belongs to the group of lipids plays a vital role in physiological functions like the synthesis of steroid hormones and bile acids and the formation of cell membranes. The high concentration of cholesterol in human blood stream due to the distortion of

its natural balance in human body can lead to various cardiovascular disease, cerebral thrombosis and atherosclerotic disorders [32]. A quick and precise determination of cholesterol concentration in blood is still an critical problem in clinical practice. Similarly vitamins are one of the most important nutrients which aids the systematic and natural working of vital biochemical processes in biological systems. The vitamin supplements in daily diet can influence the healthy growth of the human body. The B group vitamin- Pyridoxine or Vitamin B6 is the first segregated water soluble vitamin from the B group and has a significant role in maintaining physical and mental health. Vitamin B6 supports the amino acid metabolism,s ynthesis of histamine, antibodies, nuerotransmitters and red blood cells and maintains a healthy skin [33]. For a healthy life about 1.5 - 2 mg of vitamin should be included in daily diet. The determination of vitamin B6 in various food products is always a fascinating subject of study to many researchers [34]. Different analytical procedures have been reported for the detection and quantification of Vitamin B6 such as spectrophotometric analysis, chromatographic studies and electrochemical methods [35,36].

In the present investigation different modified PGEs have been employed in the electrocatalytic determination biomolecules such as cholesterol, cortisol, Vitamin B6 and morin. The modified electrodes are effectively used for the ultra-level sensing of these biomolecules in real samples

2. Review of Literature

Literature reports shows a significant number of electrochemical studies of biomolecules by making use of carbon based sensors. Li et al. modified a glassy carbon electrode (GCE) by electro-copolymerizing PPY and SWCNT onto the surface. Distinct peaks were detected for dopamine, ascorbic acid and uric acid at pH 4.0 with DPV, but it could not be directly used in

biological samples [37]. Shahrokhian and Zare-Mehrjardi developed the simultaneous detection of dopamine and ascorbic acid using a thionine – Nafion supported MWCNT-modified CPE. But the size of the electrode is large and it has not been used in real tissue samples [38]. There were several other carbon based electrode studies which detected dopamine, uric acid, and ascorbic acid. For example, a GCE modified with poly(methylene blue) (PMB) functionalized MWCNT detected three distinct peaks for ascorbic acid, uric acid, and dopamine using CV [39]. One of the other most extensively studied examples is the graphene-based glucose sensor as the determination of glucose is highly important in diagnosis of diabetes. To detect glucose, the graphene-based electrode is usually modified by glucose oxidase (GOD) in the final step of electrode preparation, i.e. casting of the solution containing GOD onto the electrode [40]. In addition to GOD, other enzymes may be immobilized onto graphene-based electrodes for construction of various enzyme biosensors, such as horseradish peroxidase (HRP), alcohol dehydrogenase (ADH), catalase and urease [41,42]. Recently, Prasannakumar et al. fabricated an alcohol biosensor using graphene-carbon nanotubes (CNTs) modified graphite electrode immobilized with ADH [43]. Zhou et al. reported the CrGO-modified GCE could be used to detect four free bases of DNA simultaneously, which was impossible with the GCE or graphite electrode [44]. Poly (eriochrome black T) film modified pencil graphite electrode was used by Chandra et al. for the determination of dopamine in presence of uric acid [45]. Saglam et al. have conducted DPV studies of eugenol at pencil graphite electrode [46].

Detection of cortisol using enzymes such as hydroxysteroid dehydrogenases (HSDs) and cytochrome P450s (CYPs) have been already documented [47, 48]. There are some interesting studies of cortisol detection techniques using metalloporphyrins and multi-walled carbon nanotubes, nanocomposite functionalized electrodes, paper-based analytical biosensor chip

designed from graphene-nanoplatelet-amphiphilic-di-block-co-polymer composite and a chemiresistor graphene oxide sensor [49,50].

Literature study shows that not many electrochemical methods are available for the analysis of morin. Electrochemically modified electrodes for determination of morin include vanadium pentoxide nanoflake film decorated glassy carbon electrode (GCE) **[**51**]**, polyvinylpyrolidone (PVP) doped carbon paste electrode, Pt electrode [52], bare glassy carbon electrode (GCE) [53] and hanging mercury drop electrode [54], Ir decorated poly(3,4-ethylenedioxythiophene) on carbon fiber paper (Ir-PEDOT/CFP) [55], 2,5-dimercapto-1,3,4-thiadazole modified carbon fiber paper (p-DMTD/CFP) [56] are also reported. An electrochemical sensing method with molecular imprinting mechanism using Pd nanoparticles-porous graphene carbon nanotubes (CNTs) / GCE has also been listed for quercetin [57] . But this method involves tiresome electrode preparation process.

In recent years optical and electrochemical biosensors have been developed for cholesterol determination. Most of the biosensors make use of different enzymes such as cholesterol oxidase, cholesterol esterase, or cytochrome P450 as sensitive and selective agents for the determination of cholesterol [58]. The wide application of enzyme based biosensors is restricted by its limited shell life, high cost of enzymes and low stability due to its sensitivity towards pH and temperature changes. The existing conventional methods, such as chromatography, colorimetry, and mass spectrometry require complex instrumentation and highly competent personnel. Therefore these time consuming and laborious methods cannot be applied for the determination of cholesterol. In 1997 Spectrophotometric determination of Vitamin B6 was reported by R. Jimé- nez-Prieto and his co-workers [59]. Fang et al introduced an arrangement of electrokinetic chromatography along with amperometric technique to determine Vitamin B6 in

the pharmaceutical industry. Söderhjelm and Lindquist studied voltammetric analysis of Vitamin B6 employing carbon paste electrodes [60]. Literature survey proves that the electrochemical analysis is always less complicated, flexible, accurate and provides quick results. Recently electrochemical sensing has influenced different areas of research including the determination of various biomolecules, due to the cost effectiveness, and possibility of a diverse range of modifications.

From the literature survey the research gaps were identified as,

- ✓ Development of highly sensitive electrochemical sensor for biomolecules.
- ✓ Easier fabrication methods of modified electrodes
- ✓ Demand for methods of high selectivity and reproducibility are still needs to be fulfilled.
- ✓ Effective electrochemical determination of analytes in real samples.

Therefore, research involving the development of sensors for the detection and quantification of biomolecules becomes one of the most germane research areas to enhance efficiency of analysis and overall benefit to humanity.

3. Objectives

- ✓ To modify Pencil graphite electrode (PGE) for electrochemical sensing of different biomolecules.
- ✓ To characterize the modified electrodes by different analytical techniques
- ✓ To optimize the experimental conditions necessary for the electroanalytical studies of the selected biomolecules.
- ✓ To validate the applicability of developed electrochemical sensors for the detection of selected biomolecules in real samples.

4. **Research work carried out**

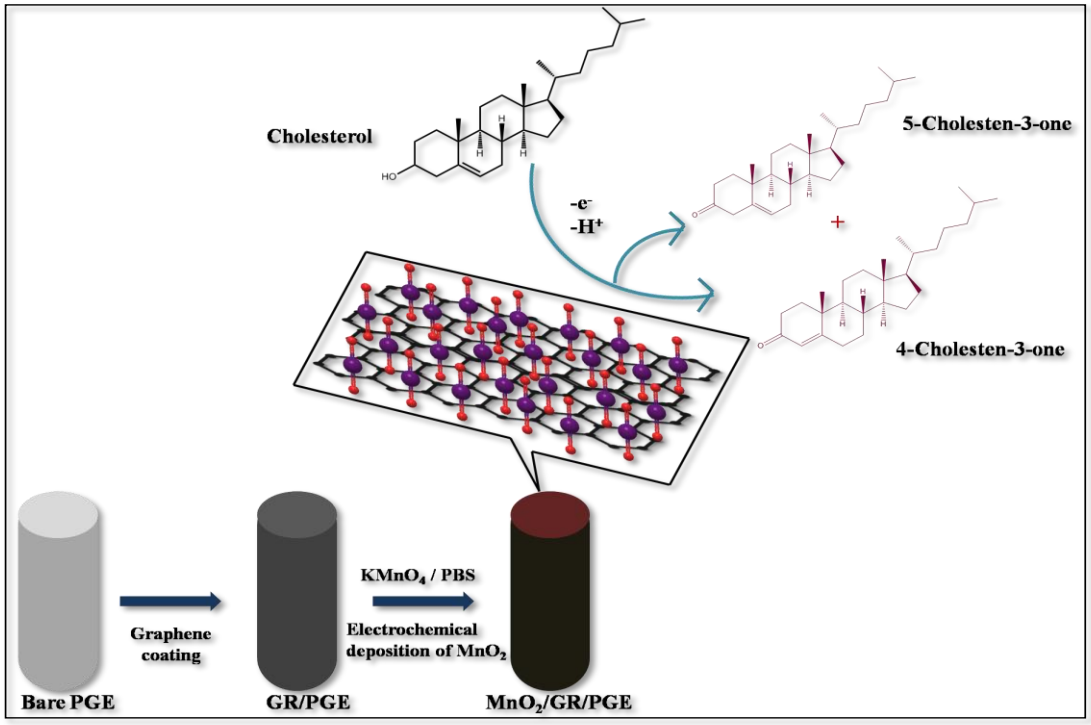
General Methodology

- ✓ Modification of substrate electrodes (PGE)
- ✓ Characterization of modified electrodes by different physicochemical -analytical methods like SEM (Scanning electron microscopy), TEM (Transmission electron microscopy), XRD (X-ray diffraction studies), XPS (X-ray photoelectronic spectroscopy) and FT-IR (Fourier transform infrared) studies.
- ✓ Optimization of experimental conditions.
- ✓ Electrochemical characterization of modified electrodes using cyclic voltammetry and differential pulsed Volta metric studies.
- ✓ Application of modified electrodes for electrochemical sensing of selected biomolecules.
- ✓ Real sample analysis by modified electrodes

Work 1- MnO_2 Nanoclusters Decorated Graphene Modified Pencil Graphite Electrode for Non-Enzymatic Determination of Cholesterol

Electrochemical deposition of MnO_2 on graphene coated PGE generates an appreciable conductive and facile electrochemical sensor for the determination of Cholesterol. Cyclic voltammetric (CV) studies and electrochemical impedance spectroscopic (EIS) technique were used to investigate the electrochemical properties of the modified sensing platform. The morphology and step wise fabrication process of the modified electrodes were characterized by X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), Transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The experimental conditions such as effect of scan rate, concentration and

pH have been optimized. The linear dynamic range for the determination of Cholesterol was estimated to be 12×10^{-10}M – 240×10^{-10}M under optimum conditions. The ultrasensitivity of the proposed method is proved by the very low level of detection limit (0.42 nM). The developed method has been successfully applied for the non-enzymatic determination of Cholesterol in human blood samples at ultralow levels. Even in the presence of other interfering substances, the proposed method displayed high selectivity towards determination of Cholesterol.



Scheme 1. Schematic illustration of electrocatalytic oxidation of Cholesterol at MnO_2/ GR/PGE.

Electrochemical deposition of MnO_2 was performed on GR/PGE electrode in 0.01M H_2SO_4 containing 25 mM $KMnO_4$ from -1.0 V to 0.8 V at 50 mVs^{-1} for 20 cycles giving the cathodic peak at 0.39 V. In neutral aqueous solution, reduction of MnO_4^- proceeds via the following reaction. The cathodic peak implies the deposition of MnO_2 at the graphene coated PGE surface.

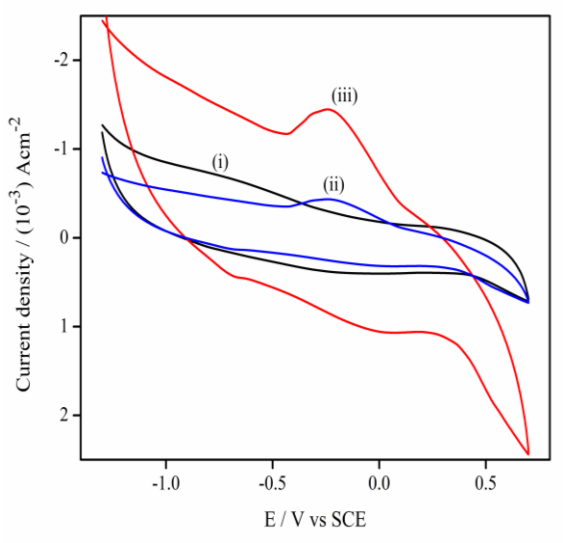


Figure 1. Cyclic voltammogram of cholesterol at (i) bare PGE, (ii) GR/PGE and (iii) MnO_2 /GR/PGE

Cholesterol showed a weak current signal at bare PGE and in comparison, MnO_2/GR/PGE has showed an evident raise in the anodic peak current at -0.24 V, indicating better electron transfer kinetics at MnO_2/GR/PGE (**Figure 1**). Metal oxide nano particles exhibit high catalytic efficiency in electrochemical reactions. The favored sites for the electrooxidation of cholesterol are C_3 –OH group, double bond between C_5 and C_6, allylic positions and tertiary positions. The surface defects and high elctroactive surface sites of MnO_2/GR/PGE attracted the reactants to the electrode surface and created an enhancement of reaction rate due to the higher current response at the modified electrode. During the oxidation of Cholesterol the hydroxyl group is converted into keto group which is well established by XPS study and the released proton. Molecules of Cholesterol were greatly attracted towards the electrode surface due to high energy surface active sites produced by graphene coating followed by MnO_2 deposition on substrate electrode.

The suitability of the method proposed was assessed by determining Cholesterol in human blood serum samples. The samples were obtained from healthy humans and processed as per the

sample preparation method as described in the experimental section. Standard addition method using DPV technique was employed for the quantification of Cholesterol content in these samples. The spiked samples of Cholesterol were diluted with PBS (pH 7.0) and only adequate amounts were used for the determination of Cholesterol. A significant oxidation peak of Cholesterol was observed around −0.22 V potential on the differential pulse voltammograms for all the real samples. The results highlight the efficiency of the proposed method in the quantitative determination of Cholesterol in human blood serum sample. A nanomolar level detection limit of Cholesterol was obtained for the first time using MnO_2/GR/PGE. The proposed method did not exert any significant effects on interfering substances in the determination of Cholesterol. This method was effectively applied to determine Cholesterol in human blood serum samples.

Work 2- β-cyclodextrin - PANI decorated pencil graphite electrode for the electrochemical sensing of morin in almonds and mulberry leaves

Morin (3,2,4,5,7-pentahydroxyflavone) is one of the natural flavonoids which is present in a variety of fruits and herbs. β-cyclodextrin (β-CD) and polyaniline (PANI) decorated Pencil graphite electrode (PGE) has been successfully used as a sensitive electrode for the determination of morin. The hydroxyl groups of β-CD attract the analyte towards the modified electrode through hydrogen bonding. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques were employed to study the electrochemical properties of the modified electrodes. The enhanced surface roughness of β-CD-PANI/PGE has resulted in the increase of electrocatalytic activity of electrode towards the analyte. Opitical profilometric studies were performed to evaluate the surface roughness of electrodes and differential pulse

votammetry (DPV) was used for the quantitative analysis of morin. Scanning electron microscopy (SEM), Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy were carried out to know the physicochemical characteristics of the modified electrodes. The experimental conditions such as scan rate, pH and concentration were optimized. The electrochemical process was found to be adsorption controlled and irreversible from scan rate studies. Under optimal conditions, the linear dynamic range for the quantification of morin was found to be 1.17 nM - 32 nM. The low detection limit (0.38 nM) indicates ultrasensitivity of the proposed method. The suggested method has been effectively employed for the estimation of morin in almonds and mulberry leaves.

Scheme 2. Schematic representation of electrocatalytic oxidation of morin at β-CD -PANI/PGE.

Fast electron transfer between morin and electrode is facilitated by β-CD-PANI modified PGE. The modified electrode was prepared by the electrodeposition of aniline and β-CD in presence of SDS and H_2SO_4 as supporting electrolyte. The hydroxyl groups present in β-CD attract morin

molecules towards the electrode surface due to the strong electrostatic interaction. The strong hydrogen bond between oxygen atom of hydroxyl group and –NH group on aniline makes β-CD-PANI modification on PGE more stable. The physicochemical studies prove the presence of hydrogen bonding in β-CD–PANI/PGE.

The electrochemical response of ferrocyanide/ferricyanide at β-CD -PANI /PGE , PANI /PGE and bare PGE were analysed at 0.05 V/s (**Figure 2**). The electroactive surface area of the electrode was estimated from the cyclic voltammetric data acquired for the redox couple. The oxidation peak current was plotted against square root of scan rate and slope was determined from the resultant graph. Randles–Sevcik equation (Equation 1) was employed to compute the surface area of the electrode from the slope value, concentration and diffusion coefficient ((6.70 ± 0.02) x 10^{-6} cm^2/s).

$$i_p = 2.69 \times 10^5 A D_o^{1/2} n^{3/2} \nu^{1/2} C \qquad (1)$$

Where, A stands for electroactive surface area in cm^2, D_o represents the diffusion coefficient in cm^2/s, n indicates the number of electrons participating in the redox reaction (n=1), ν is the scan rate in V/s, and C is the concentration of $K_4[Fe(CN)_6]$ /$K_3[Fe(CN)_6]$ in the solution in mol/cm^3. The surface area calculated for bare PGE, PANI/PGE, and β-CD-PANI/PGE were found as 1.121, 2.225 and 2.710 cm^2 respectively. The calculated surface area value of bare PGE was found lower than that of modified electrodes. These results revealed that β-CD-PANI thin layer provides enhanced surface area to PGE.

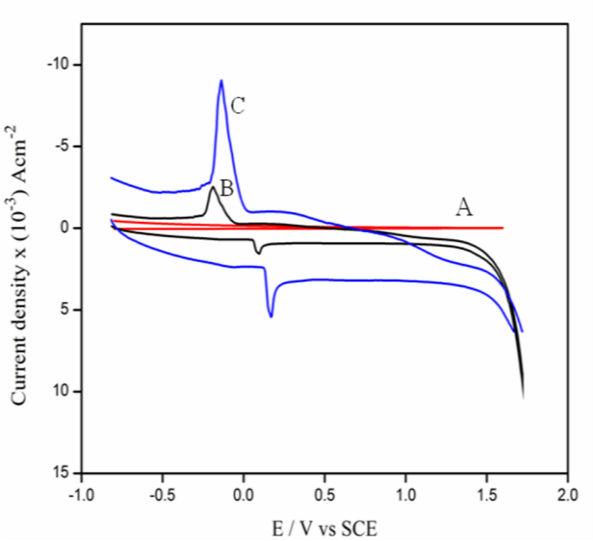


Figure 2. Cyclic voltammograms of 1mM potassium ferrocyanide/ferricyanide at bare PGE (A), GR/PGE (B), and MnO_2/GR/PGE (C) in 1 M KCl.

SEM images of bare PGE, PANI/PGE and β-CD-PANI/PGE are shown in **Figure 3**. It confirms the electrodeposition of polyaniline on the bare substrate. The electrochemically deposited polyaniline increased the electroactive surface area and PGE substrate was uniformly covered by the polymer. The presence of surfactant provided the support for the uniform development of polyaniline on substrate electrode. The SEM image clearly explains the high porous nature and increased surface defects of the β-CD-PANI/PGE compared to PANI/PGE and bare PGE. The β-CD-PANI/PGE enhanced the electrode electrolyte contact area by increasing the electroactive sites. It also provides a greater contact time for the morin molecules to get easily oxidized.

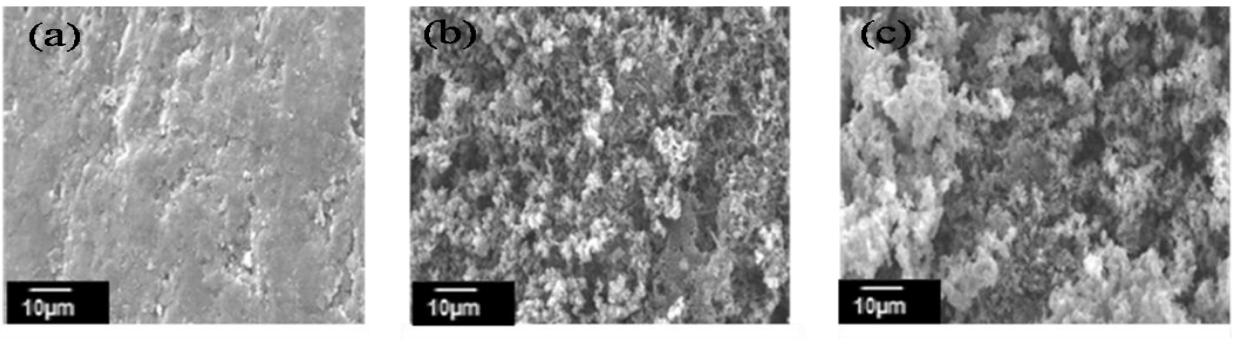


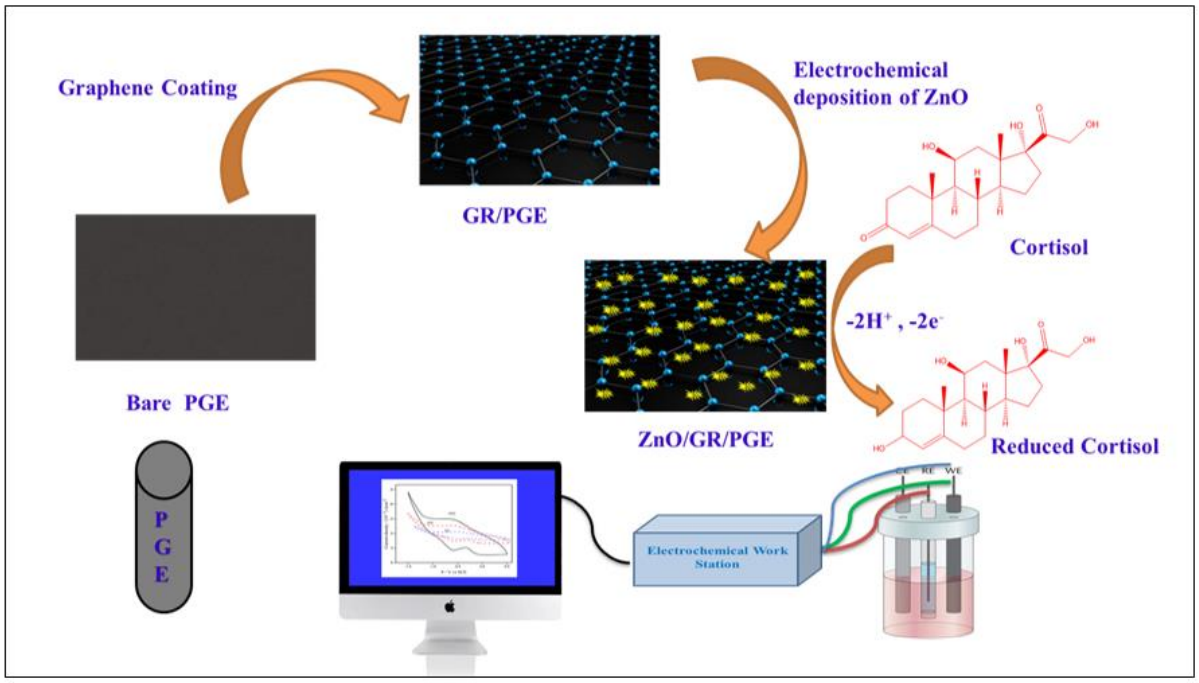
Figure 3. SEM images of bare PGE (a), PANI/PGE (b) and β-CD-PANI/PGE (c)

The feasibility of the suggested technique was evaluated by determining the analyte, morin in almonds and mulberry leaves. Differential Pulse Voltammetry was adopted to check the extraction efficiency. Experimental factors like extraction order, extraction time and volume of extractant were optimized. In DPV studies of all real samples a significant anodic peak at 0.12 V was observed. The reproducibility of the proposed method with β-CD-PANI/PGE was also investigated by analyzing morin in spiked samples having the extracts of almonds and mulberry leaves. The results revealed that the developed method was competent in the electrochemical determination of morin in real samples .

Work 3. ZnO Nanoparticles Deposited on Graphene Coated Pencil Graphite Electrode for Non-Enzymatic Determination of Cortisol

Electrochemicaly modified graphene coated PGE with ZnO generates a remarkable conductive and selective electrochemical sensing electrode for the estimation of Cortisol. Electrochemical techniques such as Cyclic voltammetric (CV) studies and electrochemical impedance spectroscopic (EIS) analysis were adopted to investigate the properties of the modified sensor. Surface morphological analysis and elemental characterization studies were conducted by X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), Transmission electron

microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The experimental conditions such as effect of scan rate, concentration and number of cycles were optimized. Differential pulse voltammetric (DPV) analysis shows that the linear range for the detection of Cortisol is5 × 10^{-10}M – 115 × 10^{-10}M with very low level limit of detection value (0.15 nM). The demonstrated method has been wonderfully functional for the non-enzymatic determination of Cortisol in human saliva samples at very trace level concentration with high selectivity even in the presence of interfering substances.



Scheme 3. Schematic representation of electrocatalytic determination of cortisol at ZnO/GR/PGE.

Cortisol estimation process was validated by measuring the linearity range, limit of detection (LOD) and limit of quantitation (LOQ). Differential Pulse Voltammetry (DPV) study showed a significant cathodic peak current at -0.58 V (**Figure 4A**). The reduction current peak value was observed to rise as the concentration of Cortisol was increased (5 × 10^{-10}M – 115 × 10^{-10}M). The

linearity calibration curve for the Cortisol quantification is exhibited in the **Figure 4B**. The linear regression equation is given as follows

i_{pa} (mA) = 3.39869E^{-4} [Cortisol] - 0.00419 (R^2 = 0.9963)

339869 μA μM^{-1} cm^{-2} was estimated as the sensitivity of the proposed method. LOD and LOQ values were calculated using the statistical formulae 3σ/S and 10σ/S respectively, where S is the slope of calibration curve and σ is the standard deviation of the peak current at any particular analyte concentration. The LOD value for the determination of Cortisol was obtained as 0.15 nM whereas the value of LOQ was found to be 0.5 nM.

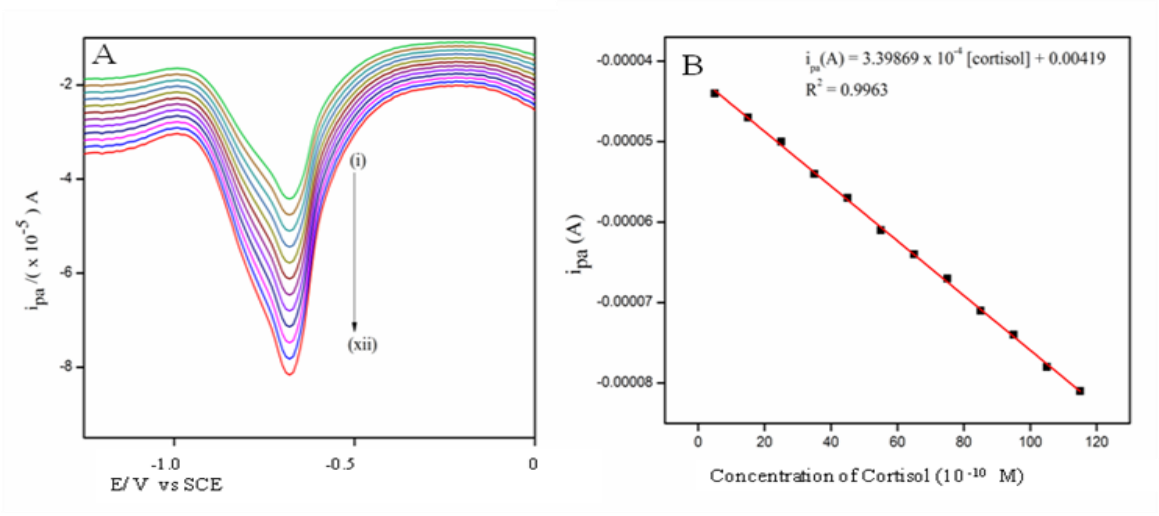


Figure 4. (A) DPV of cortisol in PBS 7 from concentration 5 × 10^{-10}M – 115 × 10^{-10}M between the potential range -1.25 V – 0 V, (B) Calibration curve of cortisol under same conditions

X-ray photoelectron spectroscopy (XPS) was used to analyze the surface chemistry of the modified electrode ZnO /GR/PGE. The survey scan XPS spectrum **Figure 5A** shows the presence of Zn 2p, C1s, and O1s on the modified electrode surface, signifying the presence of oxides of zinc on graphene. The **Figure 5B** represents the C1s with peak at 284.5 eV which point

to the sp^2 carbon system by C–C bonds in graphene. This convoluted two peaks, red and green curves stands for C=C / C-C bonds respectively. The core level spectrum of O1s proves the presence of Zn oxides, as observed in **Figure 5C**. The existence of O^{2-} and the bond between Zn and O is also confirmed by the peak at 528.7 eV. The Gaussian fitting of XPS spectra of O 1s has resulted in two peaks at 528.7 and 529.9 eV. The presence of lattice oxygen in the form of Zn-O binding attributes to the peak at 528.7 eV and the oxygen vacancy defects is indicated by the peak at 529.9 eV. **Figure 5D** of Zn 2p XPS study reveals the core level Zn $2p_{1/2}$ and Zn $2p_{3/2}$ spin–orbits peaks of ZnO, with binding energies of 1045.2 and 1022.2 eV, respectively, confirming the presence of ZnO. The spin-energy separation of 23 eV is also observed between the two peaks, which is in agreement with the documented data for Zn $2p_{1/2}$ and Zn $2p_{3/2}$ in ZnO.

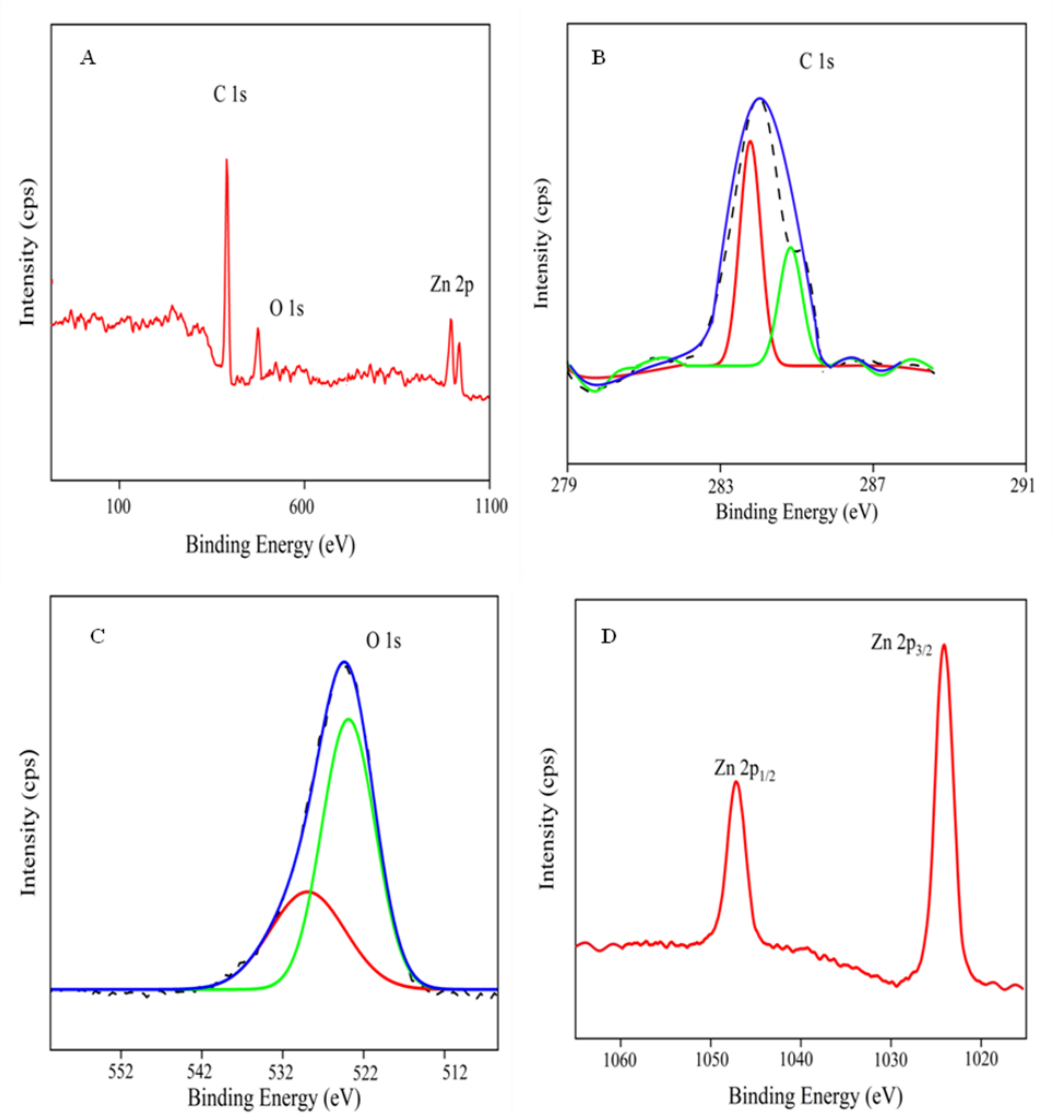
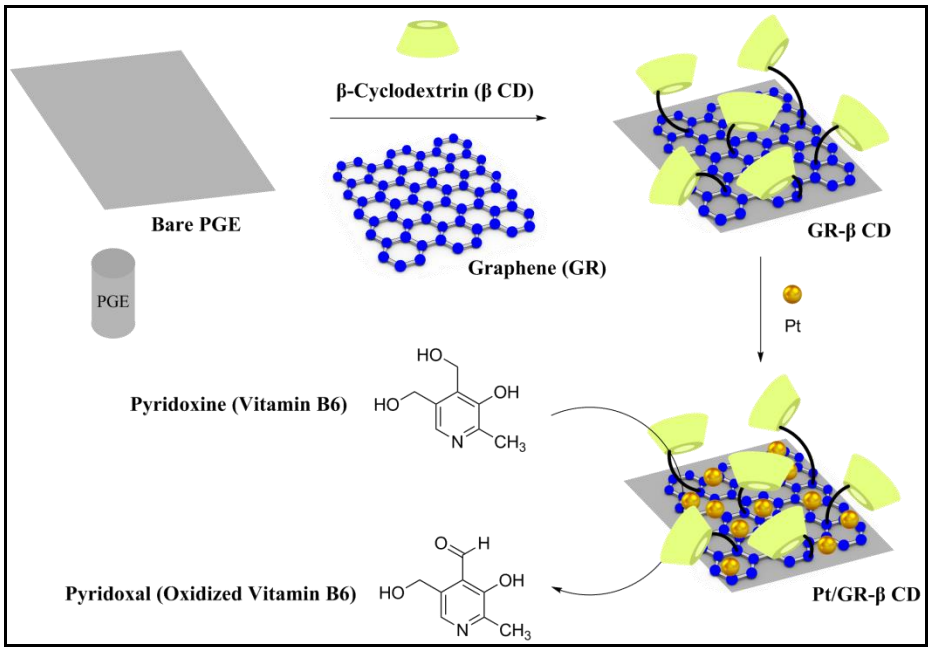


Figure 5. XPS spectra of (A) survey scan, (B) C 1s, (C) O 1s, (D) Zn 2p

The proposed method of study was validated by estimating cortisol in human saliva. Standard addition method using DPV technique was explored for the determination of Cortisol content in the real samples. The spiked samples of Cortisol were diluted with PBS (pH 7.0) and adequate portions were employed for the estimation. A significant reduction peak of Cortisol was observed around -0.58 V on the differential pulse voltammograms during the real sample analysis.

Work 4-Pt Nanospheres Decorated Graphene-βCD modified Pencil Graphite Electrode for the Electrochemical Determination of Vitamin B6

A sensitive and low detection level electrochemical sensing of Vit B6 is conducted using platinum nanoparticles decorated β-cyclodextrin-graphene modified PGE. Cyclic voltammetry was used to evaluate the electrochemical behavior of different modified electrodes towards Vit B6. The results reveal that Pt/β-CD-GR/PGE modified electrode has high electrocatalytic activity towards Vit B6 than bare electrode. Under optimum conditions, DPV analysis revealed that Pt/β-CD-GR/PGE detects vit B6 in the concentration range from 5 nM to 205 nM with high selectivity with limit of detection 1.2 nM. The physico-chemical studies of modified electrodes was investigated using X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), Transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) techniques. This method was effectively applied to determine Vit B6 in different fruit juice Samples.



Scheme 4. Schematic illustration of electrocatalytic oxidation of Vit B6 at Pt/β-CD-GR/PGE

Cyclic voltammetric technique was used for electro-deposition of Pt nanoparticles on β-CD-GR/PGE in the potential range -0.3 V – 1.4 V. The cyclic voltammogram of electro-deposition of platinum nano particles gave two redox couples at -0.046 V and -0.216 V; 1.031 V and 0.473 V which indicates the two oxidation states Pt (II) and Pt (IV) . The modified electrode Pt/β-CD-GR/PGE was then rinsed with double distilled water dried and used for the electrochemical determination of vitamin B6. Platinum nanoparticle deposition enhance the fast electron transfer between analyte and the modified electrode which facilitate electrocatalytic oxidation of Vitamin B6.

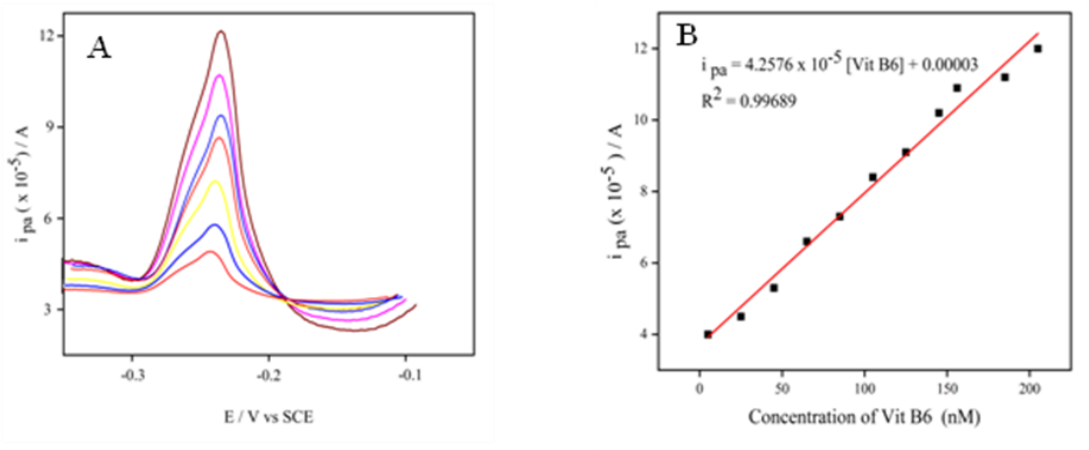


Figure 6. DPVs of Vit B6 at different concentrations from 5 nM to 205 nM in the potential range -0.35 V– 0.05 V vs SCE in PBS (pH 7.0) and Calibration plot for determination of vit B6 under same experimental conditions.

The practicability of the proposed method was studied by detecting Vit B6 in different juice samples. In DPV studies of all real samples a significant anodic peak at -0.24 V was observed. The reproducibility of the proposed method with Pt/β-CD-PGR/PGE was also investigated by analyzing Vit B6 in spiked samples. The results revealed that the developed method was competent in the electrochemical determination of Vit B6 in real samples.

5. Conclusion

Redox reactions of biomolecules at modified carbon based electrode surface is investigated using electrochemical techniques such as Cyclic Volyammetry and Differential pulsed Voltammetric analysis. PGE is used as the substrate electrode due to its cost effective nature, ease of disposability, high sensitivity and reproducibility. PGE is found to be a better substitute to other conventional expensive electrodes. The electroactive surface area and the conduvtivity of bare PGE is enhanced different electrode modifiers such as β-CD, graphene, conducting polymer, metal oxides and metal nanoparticles. The modified electrodes are found to exhibit good electrocatalytic behavior towards the target biomolecules.

MnO_2 , ZnO and Pt nanoparticles were uniformly dispersed onto graphene and β-CD graphene coated PGE to improve the electrocatalytic determination of biomolecules. The modified electrodes: MnO_2/GR/PGE, β-CD-PANI/PGE, ZnO/GR/PGE and Pt/ β-CD-GR/PGE were employed for the electrocatalytic determination of Cholesterol, Morin, Cortisol and Vit B6. The modified electrodes were characterized by FTIR, XPS, XRD, SEM, TEM, Raman, and Optical profilometric studies. Cyclic Voltammetric and Differential pulsed voltammetric analysis was used for the detection and quantification of different biomolecules. Electrochemical impedance spectroscopic analysis was adopted to investigate the improved conductivity of modified electrodes. The proposed method was effectively used for the real sample analysis in ultra trace level.

The thesis will be comprised of seven chapters. Chapter 1 and 2 will be containing Introduction and significant literature review on electrode modifications and selected biomolecules. Chapter 3 will explain the electrochemical determination of cholesterol in human blood samples using MnO_2/GR/PGE. Electrocatalytic detection of morin in mulberry leaves and almonds by β-CD-

PANI/PGE will discussed in chapter 4. ZnO deposited on GR/PGE for the electrochemical sensing of cortisol in human saliva will explained in chapter 5. Chapter 6 will discuss the electrochemical determination Vit B6 in juice sample using Pt/ β-CD-GR/PGE. Chapter 7 will contain the conclusions of whole research work and will be followed by publications and conference proceeding details.

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Publications and proceedings

1. **Papers in journal**

- Rison Sherin, K. B. Akshaya, Vinay S. Bhat, G. Shanker, T. Maiyalagan, E. K. Joice, Gurumurthy Hegde, and Anitha Varghese. "MnO2 Nanoclusters Decorated on GrapheneModified Pencil Graphite Electrode for Non- Enzymatic Determination of Cholesterol." ***Electroanalysis*** 32, (2020): 2128-2136.
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2. Conference Proceedings

1. *Poster presentation titled by " Oxidation of benzyl alcohol in aqueous medium at polythiophene deocorated with platinum nanoclusters on stainless steel substrate" in international Conference on Advances in chemical and Material Sciences held in the department of Chemistry, Mangalore University during October 17-19, 2019*
2. *Poster Presentation titled "Electrocatalytic oxidation and dtermination of morin at β-Cyclodextrin-PANI decorated graphite pencil electrode" in the 12th International Symposium on electrochemical Science and technology (iSAEST – 12) held by CECRI, Tamil Nadu during , January 08-10, 2019.*
3. *Poster presentation titled by " Benzyl alcohol oxidation in aqueous medium on stainless substrate deocorated polythiophene and platinum nanoclusters" in the international virtual Conference, Progress and promise in chemical science (PPCS -2020) during February 15, 2020.*